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Silsesquioxane polymer as a potential scaffold for laryngeal reconstruction

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ABSTRACT

Cancer, disease and trauma to the larynx and their treatment can lead to permanent loss of structures critical to voice, breathing and swallowing. Engineered partial or total laryngeal replacements would need to match the ambitious specifications of replicating functionality, outer biocompatibility, and permissiveness for an inner mucosal lining. Here we present porous polyhedral oligomeric silsesquioxane-poly(carbonate urea) urethane (POSS-PCUU) as a potential scaffold for engineering laryngeal tissue. Specifically, we employ a precipitation and porogen leaching technique for manufacturing the polymer. The polymer is chemically consistent across all sample types and produces a foam-like scaffold with two distinct topographies and an internal structure composed of nano- and micro-pores. While the highly porous internal structure of the scaffold contributes to the complex tensile behaviour of the polymer, the surface of the scaffold remains largely non-porous. The low number of pores minimise access for cells, although primary fibroblasts and epithelial cells do attach and proliferate on the polymer surface. Our data show that with a change in manufacturing protocol to produce porous polymer surfaces, POSS-PCUU may be a potential candidate for overcoming some of the limitations associated with laryngeal reconstruction and regeneration.

1. Introduction

Laryngeal cancer, disease, trauma and their treatment affect the breathing, swallowing and voice [1, 2]. Depending on the extent of the damage, the issues may be corrected by medialization, [3–5] laryngotracheal reconstruction [6], partial or total laryngectomy [7], or the problem may simply be bypassed by tracheostomy. In many, the damaged laryngeal tissue and its repair severely reduce the patient's quality of life [8, 9].

The human larynx sits at the crossroads of three critical functions: eating, breathing and talking. It has six linked cartilages, a finely controlled array of small muscles and delicate neurovascular supply [10]. Microscopically, the larynx has ciliated [11], goblet [12], brush [13], small granule [14] and basal [15] cells. Damage to any part of this complex structure can affect more than one critical function so its repair is not a simple task, often necessitating choosing one activity to preserve at the expense of the others. To rebuild the normal balanced complexity each element must be recreated: a formidable challenge.

Tissue engineering combines fundamental engineering theory with biological systems to create morphological, chemical and functional mimics of healthy tissue that allow better integration between the native and synthetic tissues [16, 17]. This approach hypothetically reduces the chances of the adverse immune responses associated with allotransplantation for example. One of the most common forms of tissue engineering involves introducing the patient's own cells onto a supportive temporary scaffold, providing them with sufficient nutrients to expand and differentiate before implantation at the injured/diseased site [18]. Selecting a scaffold material which will be conducive to cell growth requires mechanical strength, degradation behaviour and integration with surrounding tissue post-implantation [19, 20]. Encouraging seeded or infiltrating cells to attach, migrate, proliferate and differentiate is also challenging. Cell behaviour is guided by physical and chemical cues [21] and growth factors to interact with cell receptors [22, 23]. Scaffold material can lever any or all of these in a

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controlled way.

Suitable materials can be found from natural and synthetic sources [24, 25] and include, but are not limited to, polyethylene terephthalate, polytetrafluoroethylene, polyglycolic acid [26], collagen [27], gelatin [28], alginate [29], peptide-based materials [30, 31] and decellularised scaffolds [32]. However, successful implantation of these materials is still hindered by a chemical and physical mismatch between the native and engineered tissues [33]. Often materials that are mechanically stable do not encourage cell growth while materials that do encourage cell proliferation and differentiation are usually mechanically weak [34]. For this reason a combinatorial approach, bringing together two or more materials to create novel hybrid scaffolds has been proposed [35, 36]. These hybrids may be fully synthetic [37], natural [38, 39] or a mixture of the two [36].

Polyhedral oligomeric silsesquioxanes (POSS) are a candidate class of materials with cage-like structures composed of silicone and oxygen. The external surface of the POSS nanocage is composed of easily modifiable organic moieties, usually hydrocarbons [40]. Many researchers have modified these groups to create hybrid materials for a range of purposes, such as thermal and mechanical stability for circuit printing [41], thermosetting polymers [42], to remove sulphur from fuels [43] and for sea water desalination [44]. Modification of POSS-nanocage exterior has also allowed medically-relevant materials to be developed [45–48]. POSS-PCUU is a hybrid of POSS and poly(carbonate urea) urethane, a member of the widely-used polyurethane polymer family [49–51].

POSS-PCUU has been shown to have suitable mechanical properties to retain the formed geometry in vivo [52–54]. Here, we use a precipitation and porogen-leaching method to create porous scaffolds and assess the effect of increasing concentrations of porogen on material properties including chemistry, surface morphology, roughness and wettability, internal porosity, mechanical integrity and cell compatibility. While previous studies have explored the use of POSS-PCUU in vitro and in vivo, to our knowledge, this is the most comprehensive, analytical study conducted on porous POSS-PCUU to date.

2. Materials and methods

2.1. POSS-PCUU manufacture and scaffold formation

2.1.1. POSS-PCUU manufacture

All chemicals for polyhedral oligomeric silsesquioxane poly(carbonate-urea) urethane (POSS-PCUU) manufacture were purchased from Sigma Aldrich Ltd. (UK) unless stated otherwise. Briefly a polyol blend was formed by heating a mixture of *trans*-cyclohexanechloroydrinisobutyl-silsesquioxane (Hybrid Plastics, USA) and polycarbonate polyol (2000 MW) to 130 °C. After cooling the solution to 80 °C a prepolymer solution was formed by adding flake 4,40 - methylene bis(phenyl isocyanate) and heating the mixture at 70–80 °C for 120 min. To the prepolymer solution, dimethylacetamide (DMAC) was added dropwise and cooled to 35 °C. A mixture of ethylenediamine in DMAC was then added dropwise to extend the polymer chain and form POSS-PCUU. The resulting solution was stored at room temperature until use.

2.1.2. Particle size distribution and sphericity

Particle size distributions were measured using a QICPIC powder size analyzer, operated using VIBRI and GRADIS units (Sympatec, UK). Videos were analysed frame-by-frame to yield density distributions (q3) from which the following statistical parameters were calculated according to well established procedures [55–57]: x_{10}, x_{50}, x_{90}, Sauter Mean Diameter (SMD), Volume Mean Diameter (VMD), aspect ratio, and sphericity.

2.1.3. POSS-PCUU scaffold formation

Scaffold solutions were created by mixing POSS-PCUU with sodium hydrogen carbonate ($NaHCO_3$) particles sieved at 25-53 μm (or 53-100 μm where stated; Fig. S1). Tween 20 was also added at 2% (w/w). The ratio of $NaHCO_3$ to POSS-PCUU for each sample is shown in Table 1.

Table 1
Quantity of $NaHCO_3$ in POSS-PCUU and DMAC for each sample type.

Sample	$NaHCO_3$ in POSS-PCUU + DMAC (% w/w)	$NaHCO_3$ in POSS-PCUU (% w/w)
1	0	0
2	1	5.7
3	5	24
4	10	40.1
5	20	60.1
6	30	72.2
7	40	80.2
8	50	86
9	50 (53-100 μm)	86

The solution was mixed using a centrifugal mixer (2000 rpm, Thinky ARE-250, USA) and any air bubbles removed through a 'de-gassing' cycle (1500 rpm) on the mixer. The mixed solution was then poured onto a clean glass mould measuring 148 × 210 mm which was surrounded by a ~300 μm thick autoclave tape perimeter. To precipitate the polymer and allow $NaHCO_3$ particles to leach out of the scaffold the glass mould was slowly submerged into a bath containing 5 L of deionised water (DI H_2O; Fig. 1). The DI H_2O was replaced 3 times a day for 5 days before the precipitated porous polymer was peeled off the glass mould and stored in 70% ethanol (v/v ethanol in water). For polymer characterisation studies the scaffold was first washed overnight in DI H_2O while for cellular studies the samples were autoclaved at 121 °C for 20 mins in DI H_2O before use (Fig. S2). For the purpose of this study we refer to precipitated polymer as 'scaffold'.

2.2. Material characterisation

2.2.1. Raman spectroscopy

The surface chemical composition of the precipitated POSS-PCUU polymers was evaluated using a confocal Raman microscope (WiTec Alpha 300R, LOT Oriel, UK) with a 0.3 W single frequency 785 nm diode laser (Toptica Photonics, Germany). Data were collected via an Acton SP2300 triple grating monochromator/spectrograph (Princeton Instruments, USA) over a 200–3000 cm^{-1} wavenumber range at a mean spectral resolution of 3 cm^{-1}.

2.2.2. X-ray photoelectron spectroscopy

The composition and chemical bonds of the polymer surface were investigated using X-ray photoelectron spectroscopy (XPS). Analysis was performed using a K-alpha X-ray Photoelectron Spectrometer (Thermo Fisher Scientific, UK), operating a microfused, monochromated Al Kα X-ray source with a spot size of 400 μm and a power of 36 W. The step size in order to obtain individual peaks was 0.1 eV, whereas 1 eV was used for the acquisition of a full spectrum over the complete range of binding energies (BEs). The vacuum pressure in the analysis chamber was $< 10^{-9}$ mbar. The C 1s (BE = 285 eV), N 1s (BE = 400 eV) and O 1s (BE = 531 eV) photoelectron peaks were analysed in detail. Three non-overlapping regions were measured for each sample. Data were fitted using the Gauss-Lorentz function, and the Shirley method was used for background subtraction. CasaXPS software was used for data processing.

2.2.3. Field emission scanning electron microscopy

The microstructure of precipitated POSS-PCUU polymer samples was evaluated by field emission scanning electron microscopy (FEG-SEM, Quanta 200F, FEI, USA). The samples were first dehydrated in graded ethanol 20%, 30%, 50%, 70%, 90% and 100% v/v ethanol in